REISSUE PATENT
Docket No. 77670/495

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors	:	Hiroyuki NAKANE Chikara OHTO Shinichi OHNUMA Kazutake HIROOKA Tokuzo NISHINO	
Reissue Appln. No.	:	To Be Assigned	(Reissue of 08/898,560, filed on July 22, 1997, now U.S. Pat. No. 5,935,832, issued August 10, 1999)
Reissue Filing Date	:	Herewith	
For	:	FARNESYL DIPHOSPHATE SYNTHASE	
Group Art Unit	:	To Be Assigned	
Examiner	:	To Be Assigned	

BOX REISSUE
COMMISSIONER FOR PATENTS
Washington, DC 20231

PRELIMINARY AMENDMENT

S I R:

Applicants respectfully request that this Preliminary Amendment be entered in the above-identified broadening reissue application before examination of said application.

IN THE SPECIFICATION

At col. 1, line 15, please delete the "s" from "unit[s]". The paragraph containing this text is reproduced below.

Of the substances having important functions in organisms, many are biosynthesized using isoprene (2-methyl-1,3-butadiene) as a constituent unit. These

compounds are also called isoprenoids, terpenoids, or terpenes, and are classified depending on the number of carbon atoms into hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), tetraterpenes (C40), and the like. The actual biosynthesis starts with the mevalonate pathway through which mevalonic acid-5-diphosphate is synthesized, followed by the synthesis of isopentenyl diphosphate (IPP) which is an active isoprene unit.

At col. 1, line 45, please delete the "plants" and at col. 1, line 46, after "in" please insert "plants." The paragraph containing this text is reproduced below.

There are Z type and E type condensation reactions. Geranyl diphosphate is a product of E type condensation and neryl diphosphate is of Z type condensation. Although, the all-E type is considered to be the active form in farnesyl diphosphate and geranylgeranyl diphosphate, the Z type condensation reaction leads to the synthesis of natural rubber, dolichols, bactoprenols (undecaprenols), and various polyprenols found in plants. They are believed to undergo the condensation reaction using the phosphate ester bond energy of the pyrophosphate and the carbon backbone present in the molecule and to produce pyrophosphate as the byproduct of the reaction.

At col. 2, line 4, please delete "[geraniols and that isomer nerol belonging]" and substitute "geraniol and its isomer, nerol, belonging " therefor. At col. 2, line 5, after "monoterpens" please insert "that." The paragraph containing this text is reproduced below.

Furthermore, via the biosynthesis of these active-form isoprenoids, a vast number of kinds of compounds that are vital to life have been synthesized. Just to mention a few, there are cytokinins that are plant hormones and isopentenyl adenosine-modified tRNA that use hemiterpenes as their precursor of synthesis, geraniol and its isomer, nerol, belonging to monoterpens that are the main components of rose oil perfume and a camphor tree extract, camphor, which is an insecticide. Sesquihormones include juvenile hormones of insects, diterpenes include a plant hormone gibberellin, trail pheromones of insects, and retinols and retinals that function as the visual pigment precursors, binding components of the purple membrane proteins of highly halophilic archaea, and vitamin A.

At col. 3, line 45, please delete "[(DDXX(XX)D)]" and substitute "$(D_1D_2X_1(X_2X_3)X_4D_3)$" therefore. At col. 3, line 56, please delete the "[have not been]" and substitute "are" therefor. At col. 3, line 57, please delete the "[that induce mutation]" and substitute "that include mutations" therefor. At col. 3, lines 58-59, please delete "[to be in the short chain-length side]" and substitute with having a shorter chain length. The paragraph containing this text is reproduced below.

It has been found out that of the two aspartic acid-rich domains that have been proposed based on the amino acid sequence of the prenyl diphosphate synthase, the amino acid residue located at the fifth position in the N-terminal direction from the conserved sequence I ($D_1D_2X_1(X_2X_3)X_4D_3$) (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) of the aspartic acid-rich domain in the amino-terminal side is responsible for controlling the chain length of the reaction product. Hence, a method has been invented that controls the reaction product for the purpose of lengthening the chain length of the reaction product [Japanese patent

application No. 8-191635 filed on Jul. 3, 1996 under the title of "A Mutant Prenyl Diphosphate Synthase"]. The enzyme produced using the method enables production of reaction products that have several chain lengths. However, methods are not known that include mutation of geranylgeranyl diphosphate synthase to control the reaction products having a shorter chain length in order to produce farnesyl diphosphate.

At col. 4, line 2, please delete the "[owned by the]" and substitute "exhibited by" therefor. The paragraph containing this text is reproduced below.

It is an object of the invention to establish a process for producing farnesyl diphosphate synthases by modifying amino acid sequences of prenyl diphosphate enzymes. A new enzyme that is more stable or that has a high specific activity more adaptable to industrial application would make it possible to obtain immediately a mutant prenyl diphosphate synthase or the gene thereof that produces farnesyl diphosphate and that retains the property exhibited by the prenyl diphosphate synthase prior to mutation.

At col. 4, line 11, please delete "[(DDXX(XX)D)]" and substitute "$(D_1D_2X_1(X_2X_3)X_4D_3)$" therefore. The paragraph containing this text is reproduced below.

From the information on the nucleotide sequence of the gene of the geranylgeranyl diphosphate synthase of the mutant Sulfolobus acidocaldarius (S. acidocaldarius), it was clarified that out of the two Aspartic acid-rich domains that have been proposed based on the analysis of the amino acid sequence of prenyl

diphosphate synthases, the amino acid residues within the aspartic acid-rich domain conserved sequence I ($D_1D_2X_1(X_2X_3)X_4D_3$) at the amino terminal side or the five amino acid residues to the N-terminal side from the amino terminal of said conserved sequence I are involved in the control of chain length of the reaction products.

At col. 4, line 23, please delete "[(DDXX(XX)D)]" and substitute "($\underline{D_1D_2X_1(X_2X_3)X_4D_3}$)" therefor. At col. 4, line 26, please delete "[at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain]" and substitute "<u>one amino acid position downstream of D2</u>" therefor. The paragraph containing this text is reproduced below.

At least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain ($D_1D_2X_1(X_2X_3)X_4D_3$) (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located one amino acid position downstream of D2 has been substituted by another amino acid, and/or

At col. 4, line 32, please delete "[amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal]" and substitute "<u>first amino acid downstream of D_2 and the first amino acid upstream of D_3</u> " therefor. The paragraph containing this text is reproduced below.

Additional amino acid(s) have been inserted in between the first amino acid downstream of D_2 and the first amino acid upstream of D_3 of said aspartic acid-rich domain.

At col. 5, line 36, please delete "[(DXX(XX)D)]" and substitute "$\underline{(D_1D_2X_1(X_2X_3)X_4D_3)}$" therefore. The paragraph containing this text is reproduced below.

It has been proposed that there are five conserved regions in the amino acid sequence of a prenyl diphosphate synthase (one subunit in the case of a heterodimer) [A. Chem et al., Protein Science Vol. 3, pp. 600-607, 1994]. It is also known that of the five conserved regions, there is an aspartic acid-rich domain conserved sequence I $D_1D_2X_1(X_2X_3)X_4D_3$ (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) in region II. Although there is also an aspartic acid-rich domain indicated as "DDXXD" in region V, the aspartic acid-rich domain used to specify the modified region of the amino acid sequence of the present invention is the one present in region II, and this domain is termed as the aspartic acid-rich domain I as compared to the aspartic acid-rich domain II present in region V.

At col. 6, line 6, please delete "[(DDXX(XX)D)]" and substitute "$\underline{(D_1D_2X_1(X_2X_3)X_4D_3)}$" therefore. At col. 6, line 8, please delete "[at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain]" and substitute "<u>one amino acid position downstream of D2</u>" therefor. The paragraph containing this text is reproduced below.

In accordance with the present invention, in the amino acid sequence of a prenyl diphosphate synthase, at least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain $D_1D_2X_1(X_2X_3)X_4D_3$ (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located one amino acid position downstream of D2 has been substituted by another amino acid, and/or

At col. 6, line 16, lease delete "[amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal]" and substitute "<u>first amino acid downstream of D_2 and the first amino acid upstream of D_3</u> " therefor. The paragraph containing this text is reproduced below.

An additional amino acid(s) have been inserted in between the first amino acid downstream of D_2 and the first amino acid upstream of D_3 of said aspartic acid-rich domain.

At col. 7, line 67, please delete "[biding]" and substitute "<u>binding</u>" therefor. The paragraph containing this text is reproduced below.

It is known that the distance between the sequence of the ribosome binding site (GGAGG and similar sequences thereof) and the initiation codon ATG is important as the sequence regulating the ability of synthesizing protein from mRNA. It is also well known that a terminator (for example, a vector containing rrn PT_1 T_2 is

commercially available from Pharmacia) that directs transcription termination at the 3'-end affects the efficiency of protein synthesis by a recombinant.

At col. 9, line 14, please delete "[a]" and substitute "an" therefor. At col. 9, line 16, please delete "[prrenyl]" and substitute "prenyl" therefor. The paragraph containing this text is reproduced below.

By using the method of producing the mutant prenyl diphosphate synthase obtained by the present invention, the mutant prenyl diphosphate synthase derived from an archaea may be created that is more stable and thus easier to handle and that produces prenyl diphosphate. Furthermore, there is also expected a creation of the farnesyl diphosphate-producing mutant prenyl diphosphate synthase that has the property of the prenyl diphosphate synthase prior to mutation (for example, salt stability or stability in a wide range of pH) added thereto.

At col. 10, line 4, please delete "[Geranylaeranyl]" and substitute "Geranylgeranyl" therefor. This text is reproduced below.

Construction of a Plasmid Containing the Gene for Geranylgeranyl Diphosphate Synthase

At col. 10, line 57, please delete "[TATT-31]" and substitute "TATT-3' " therefor. The paragraph containing this text is reproduced below.

Introduction of the mutation (F77Y, T78S, V80I, I84L, 84PS85) was effected using two nucleotides. First, mutation was introduced as mentioned in Example 3

using the oligonucleotide

5'-GTTCTTCATACTTATTCGCTTATTCATGATAG TATT-3' (SEQ ID No: 7) and a transformant was prepared in accordance with Example 4, and furthermore mutation was introduced into the plasmid thus obtained using the oligonucleotide

5'-ATTCATGATGATCTTCCATCGATGGATCAAGAT-3' (SEQ ID No: 8).

At col. 11, line 24, please delete "[H2O]" and substitute "$\underline{H_2O}$ " therefor. The paragraph containing this text is reproduced below.

H_2O 5 µl

At col. 11, line 56, please delete "[H2O]" and substitute "$\underline{H_2O}$ " therefor. The paragraph containing this text is reproduced below.

H_2O make to a final volume of 10 µl

At col. 12, line 35, please delete "[ATATCATG-31]" and substitute "ATATCATG-3' " therefor. The paragraph containing this text is reproduced.

F77Y, T78F, H81L: 5'-TATTTCCTTGTGCTTGATG ATATCATG-3' (SEQ ID No: 11)

IN THE CLAIMS

Please amend claim 1 as follows:

1. A mutant prenyl diphosphate synthase having a modified amino acid sequence, wherein

said mutant diphosphate synthase comprises an aspartic acid-rich domain having the sequence, $D_1D_2X_1X_2(X_3X_4)D_3$, in region II of said mutant prenyl diphosphate synthase,

wherein each of D_1, D_2, and D_3 denote an aspartic acid residue; X_1, X_2, X_3, and X_4 are each independently any amino acid and X_3 and X_4 are each optionally independently present in the aspartic acid rich domain, and wherein

said mutant prenyl disphosphate synthase comprises (1) at least one amino acid substitution, said at least one amino acid substitution located at at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located one amino acid positions upstream of D_3; (2) at least one additional amino acid inserted between D_3 and the first amino acid upstream of D_3; or (3) a combination of (1) and (2);

wherein said mutant prenyl diphosphate synthase synthesizes prenyl diphosphate which is shorter than prenyl diphosphate synthesized by a corresponding wild-type enzyme.

Please add the following new claims:

Claim 19. A mutant prenyl diphosphate synthase having a modified amino acid sequence, wherein

said mutant diphosphate synthase comprises an aspartic acid-rich domain having the sequence, $D_1D_2X_1(X_2X_3)X_4D_3$, in region II of said mutant prenyl diphosphate synthase,

wherein each of D_1, D_2, and D_3 denote an aspartic acid residue; X_1, X_2,

X_3, and X_4 are each independently any amino acid and X_2 and X_3 are each optionally independently present in the aspartic acid rich domain, and wherein

said mutant prenyl disphosphate synthase comprises (1) at least one amino acid substitution, said at least one amino acid substitution located at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located one amino acid position downstream of D_2; (2) at least one additional amino acid inserted between the first amino acid downstream of D_2 and the first amino acid upstream of D_3; or (3) a combination of (1) and (2);

wherein said mutant prenyl diphosphate synthase synthesizes prenyl diphosphate which is shorter than prenyl diphosphate synthesized by a corresponding wild-type enzyme.

Claim 20. A mutant prenyl diphospate synthase according to claim 19 wherein said mutant has the enzymatic activities and thermostability of wild type prenyl diphosphate synthase.

Claim 21. A mutant enzyme according to claim 19 wherein the reaction product of the prenyl diphosphate synthase is farnesyl diphosphate.

Claim 22. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is a homodimer.

Claim 23. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is derived from archaea.

Claim 24. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is derived from *Sulfolobus acidocaldarius*.

Claim 25. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is a thermostable enzyme.

Claim 26. A mutant prenyl diphosphate synthase according to claim 19, wherein at least one amino acid selected from phenylalanine at position 77, threonine at position 78, valine at position 80, histidine at position 81, and isoleucine at position 84 has been substituted by another amino acid, or one or more amino acids have been inserted in between isoleucine at position 84 and methionine at position 85 in the geranylgeranyl diphosphate synthase as set forth in SEQ ID No:1.

Claim 27. A mutant prenyl diphosphate synthase according to claim 19 wherein at least one amino acid selected from phenylalanine at position 77, threonine at position 78, valine at position 80, histidine at position 81, and isoleucine at position 84 has been substituted by another amino acid, and/or two amino acids have been inserted between isoleucine at position 84 and methionine at position 85 in the geranylgeranyl diphosphate synthase as set forth in SEQ ID No: 1, wherein the phenyl alanine at position 77 has been replaced with tyrosine, the threonine at position 78 has been replaced with phenylalanine or serine, the valine at position 80 has been replaced with isoleucine, the histidine at position 81 has been replaced with leucine or alanine, or the isoleucine at position 84 has been replaced with leucine; or proline and serine

have been inserted in between the isoleucine at position 84 and the methionine at position 85.

Claim 28. A mutant prenyl diphosphate synthase according to claim 19, wherein the mutant prenyl diphosphate synthase is derived from a native geranylgeranyl diphosphate synthase of an organism selected from the group consisting of *Arabidopsis thaliana, Lupinas albus, Capsicum annuum, Sulfolobus acidocaldarius, Rhodobactor sphaeroides, Rhodobacter capsulatus, Erwinia herbicola, Myxococcus thaliana and Neurospora crassa.*

Claim 29. A DNA encoding an enzyme according to claim 19.

Claim 30. An RNA transcribed from a DNA according to claim 29.

Claim 31. A recombinant vector comprising a DNA according to claim 29.

Claim 32. A host organism transformed with a recombinant vector according to claim 31.

Claim 33. A process for producing a mutant enzyme according to claim 19, said method comprising the steps of culturing a host transformed with an expression vector comprising a DNA coding for the mutant enzyme and harvesting the expression product from the culture.

Claim 34. A process for producing a prenyl diphosphate having not more than 15 carbons comprising the step of bringing an enzyme according to claim 19 into contact

with a substrate selected from the group consisting of isopentenyl diphosphate, dimethylallyl diphosphate, and geranyl diphosphate.

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REMARKS

The present application is a broadening reissue of U.S. Appln. No. 08/898,560, filed on July 22, 1997, now U.S. Pat. No. 5,935,832 ("the '832 patent.") The '832 patent is at least partly inoperative by reason of the patentee claiming less than he had the right to claim in the patent. Support for the amendments made herein is described in the enclosed and accompanying document entitled, "Reissue Application of U.S. Patent No. 5,835,832" which also includes the inventor's oath and declaration. No new matter has been added.

The examination of the present application is requested herewith. The Examiner is invited to contact the undersigned if there are any questions regarding this Preliminary Amendment or the reissue application itself.

The Office is authorized to charge any additional fees or credit any overpayments under 37 C.F.R. § 1.16 OR 1.17 to Deposit Account No. 11-0600.

Respectfully submitted,

Dated: July 12, 2001

By: _____
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MARKED-UP VERSION OF THE CLAIMS AND SPECIFICATION CHANGES

Claims 1 has been amended as follows:

1. A mutant prenyl diphosphate synthase having a modified amino acid sequence, wherein

said mutant diphosphate synthase comprises an aspartic acid-rich domain having the sequence, $D_1D_2X_1X_2(X_3X_4)D_3$, in region II of said mutant prenyl diphosphate synthase,

wherein each of D_1, D_2, and D_3 denote an aspartic acid residue; X_1, X_2, X_3, and X_4 are each independently any amino acid and X_3 and X_4 are each optionally independently present in the aspartic acid rich domain, and wherein

said mutant prenyl disphosphate synthase comprises (1) at least one amino acid substitution, said at least one amino acid substitution located at at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located one amino acid positions upstream of D_3; (2) at least one additional amino acid inserted between D_3 and the first amino acid upstream of D_3; or <u>(3)</u> a combination of <u>(1)</u> [(2)] and <u>(2)</u> [(3)];

wherein said mutant prenyl diphosphate synthase synthesizes prenyl diphosphate which is shorter than prenyl diphosphate synthesized by a corresponding wild-type enzyme.

New claims 19-34 have been added.

Claim 19. A mutant prenyl diphosphate synthase having a modified amino acid sequence, wherein

said mutant diphosphate synthase comprises an aspartic acid-rich domain having the sequence, $D_1D_2X_1(X_2X_3)X_4D_3$, in region II of said mutant prenyl diphosphate synthase,

wherein each of D_1, D_2, and D_3 denote an aspartic acid residue; X_1, X_2,

X_3, and X_4 are each independently any amino acid and X_2 and X_3 are each optionally independently present in the aspartic acid rich domain, and wherein

said mutant prenyl disphosphate synthase comprises (1) at least one amino acid substitution, said at least one amino acid substitution located at least one amino acid position selected from (a) an amino acid between D_1 and the amino acid residue at the fifth position upstream of D_1 and (b) the amino acid residue located one amino acid position downstream of D_2; (2) at least one additional amino acid inserted between the first amino acid downstream of D_2 and the first amino acid upstream of D_3; or (3) a combination of (1) and (2);

wherein said mutant prenyl diphosphate synthase synthesizes prenyl diphosphate which is shorter than prenyl diphosphate synthesized by a corresponding wild-type enzyme.

Claim 20. A mutant prenyl diphospate synthase according to claim 19 wherein said mutant has the enzymatic activities and thermostability of wild type prenyl diphosphate synthase.

Claim 21. A mutant enzyme according to claim 19 wherein the reaction product of the prenyl diphosphate synthase is farnesyl diphosphate.

Claim 22. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is a homodimer.

Claim 23. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is derived from archaea.

Claim 24. A mutant enzyme according to claim 19 wherein the prenyl diphosphate synthase is derived from *Sulfolobus acidocaldarius*.

Claim 25. A mutant enzyme according to claim 19 wherein the prenyl diphosphate

synthase is a thermostable enzyme.

Claim 26. A mutant prenyl diphosphate synthase according to claim 19, wherein at least one amino acid selected from phenylalanine at position 77, threonine at position 78, valine at position 80, histidine at position 81, and isoleucine at position 84 has been substituted by another amino acid, or one or more amino acids have been inserted in between isoleucine at position 84 and methionine at position 85 in the geranylgeranyl diphosphate synthase as set forth in SEQ ID No:1.

Claim 27. A mutant prenyl diphosphate synthase according to claim 19 wherein at least one amino acid selected from phenylalanine at position 77, threonine at position 78, valine at position 80, histidine at position 81, and isoleucine at position 84 has been substituted by another amino acid, and/or two amino acids have been inserted between isoleucine at position 84 and methionine at position 85 in the geranylgeranyl diphosphate synthase as set forth in SEQ ID No: 1, wherein the phenyl alanine at position 77 has been replaced with tyrosine, the threonine at position 78 has been replaced with phenylalanine or serine, the valine at position 80 has been replaced with isoleucine, the histidine at position 81 has been replaced with leucine or alanine, or the isoleucine at position 84 has been replaced with leucine; or proline and serine have been inserted in between the isoleucine at position 84 and the methionine at position 85.

Claim 28. A mutant prenyl diphosphate synthase according to claim 19, wherein the mutant prenyl diphosphate synthase is derived from a native geranylgeranyl diphosphate synthase of an organism selected from the group consisting of *Arabidopsis thaliana, Lupinas albus, Capsicum annuum, Sulfolobus acidocaldarius, Rhodobactor sphaeroides, Rhodobacter capsulatus, Erwinia herbicola, Myxococcus thaliana and Neurospora crassa*.

Claim 29. A DNA encoding an enzyme according to claim 19.

Claim 30. An RNA transcribed from a DNA according to claim 29.

Claim 31. A recombinant vector comprising a DNA according to claim 29.

Claim 32. A host organism transformed with a recombinant vector according to claim 31.

Claim 33. A process for producing a mutant enzyme according to claim 19, said method comprising the steps of culturing a host transformed with an expression vector comprising a DNA coding for the mutant enzyme and harvesting the expression product from the culture.

Claim 34. A process for producing a prenyl diphosphate having not more than 15 carbons comprising the step of bringing an enzyme according to claim 19 into contact with a substrate selected from the group consisting of isopentenyl diphosphate, dimethylallyl diphosphate, and geranyl diphosphate.

The specification has been amended as follows:

At col. 1, line 15, please delete the "s" from "unit[s]". The paragraph containing this text is reproduced below.

Of the substances having important functions in organisms, many are biosynthesized using isoprene (2-methyl-1,3-butadiene) as a constituent unit. These compounds are also called isoprenoids, terpenoids, or terpenes, and are classified depending on the number of carbon atoms into hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), tetraterpenes (C40), and the like. The actual biosynthesis starts with the mevalonate pathway through which mevalonic acid-5-diphosphate is synthesized, followed by the synthesis of isopentenyl diphosphate (IPP) which is an active isoprene unit.

At col. 1, line 45, please delete the "plants" and at col. 1, line 46, after "in" please insert "<u>plants</u>." The paragraph containing this text is reproduced below.

There are Z type and E type condensation reactions. Geranyl diphosphate is a product of E type condensation and neryl diphosphate is of Z type condensation. Although, the all-E type is considered to be the active form in farnesyl diphosphate

and geranylgeranyl diphosphate, the Z type condensation reaction leads to the synthesis of natural rubber, dolichols, bactoprenols (undecaprenols), and various polyprenols found in plants. They are believed to undergo the condensation reaction using the phosphate ester bond energy of the pyrophosphate and the carbon backbone present in the molecule and to produce pyrophosphate as the byproduct of the reaction.

At col. 2, line 4, please delete "[geraniols and that isomer nerol belonging]" and substitute "geraniol and its isomer, nerol, belonging " therefor. At col. 2, line 5, after "monoterpens" please insert "that." The paragraph containing this text is reproduced below.

Furthermore, via the biosynthesis of these active-form isoprenoids, a vast number of kinds of compounds that are vital to life have been synthesized. Just to mention a few, there are cytokinins that are plant hormones and isopentenyl adenosine-modified tRNA that use hemiterpenes as their precursor of synthesis, geraniol and its isomer, nerol, belonging to monoterpens that are the main components of rose oil perfume and a camphor tree extract, camphor, which is an insecticide. Sesquihormones include juvenile hormones of insects, diterpenes include a plant hormone gibberellin, trail pheromones of insects, and retinols and retinals that function as the visual pigment precursors, binding components of the purple membrane proteins of highly halophilic archaea, and vitamin A.

At col. 3, line 45, please delete "[(DDXX(XX)D)]" and substitute "($D_1D_2X_1(X_2X_3)X_4D_3$)" therefore. At col. 3, line 56, please delete the "[have not been]" and substitute "are" therefor. At col. 3, line 57, please delete the "[that induce mutation]" and substitute "that include mutations" therefor. At col. 3, lines 58-59, please delete "[to be in the short chain-length side]" and substitute with having a shorter chain length. The paragraph containing this text is reproduced below.

It has been found out that of the two aspartic acid-rich domains that have been proposed based on the amino acid sequence of the prenyl diphosphate synthase, the amino acid residue located at the fifth position in the N-terminal direction from the conserved sequence I ($D_1D_2X_1(X_2X_3)X_4D_3$) (wherein X denotes any amino acid, and

the two X's in the parentheses may not be present) of the aspartic acid-rich domain in the amino-terminal side is responsible for controlling the chain length of the reaction product. Hence, a method has been invented that controls the reaction product for the purpose of lengthening the chain length of the reaction product [Japanese patent application No. 8-191635 filed on Jul. 3, 1996 under the title of "A Mutant Prenyl Diphosphate Synthase"]. The enzyme produced using the method enables production of reaction products that have several chain lengths. However, methods are not known that include mutation of geranylgeranyl diphosphate synthase to control the reaction products having a shorter chain length in order to produce farnesyl diphosphate.

At col. 4, line 2, please delete the "[owned by the]" and substitute "exhibited by" therefor. The paragraph containing this text is reproduced below.

It is an object of the invention to establish a process for producing farnesyl diphosphate synthases by modifying amino acid sequences of prenyl diphosphate enzymes. A new enzyme that is more stable or that has a high specific activity more adaptable to industrial application would make it possible to obtain immediately a mutant prenyl diphosphate synthase or the gene thereof that produces farnesyl diphosphate and that retains the property exhibited by the prenyl diphosphate synthase prior to mutation.

At col. 4, line 11, please delete "[(DDXX(XX)D)]" and substitute "($D_1D_2X_1(X_2X_3)X_4D_3$)" therefore. The paragraph containing this text is reproduced below.

From the information on the nucleotide sequence of the gene of the geranylgeranyl diphosphate synthase of the mutant Sulfolobus acidocaldarius (S. acidocaldarius), it was clarified that out of the two Aspartic acid-rich domains that have been proposed based on the analysis of the amino acid sequence of prenyl diphosphate synthases, the amino acid residues within the aspartic acid-rich domain conserved sequence I ($D_1D_2X_1(X_2X_3)X_4D_3$) at the amino terminal side or the five amino acid residues to the N-terminal side from the amino terminal of said conserved sequence I are involved in the control of chain length of the reaction products.

At col. 4, line 23, please delete "[(DDXX(XX)D)]" and substitute "$\underline{(D_1D_2X_1(X_2X_3)X_4D_3)}$" therefor. At col. 4, line 26, please delete "[at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain]" and substitute "one amino acid position downstream of D2" therefor. The paragraph containing this text is reproduced below.

At least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain $(D_1D_2X_1(X_2X_3)X_4D_3)$ (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located one amino acid position downstream of D2 has been substituted by another amino acid, and/or

At col. 4, line 32, please delete "[amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal]" and substitute "first amino acid downstream of D_2 and the first amino acid upstream of D_3 " therefor. The paragraph containing this text is reproduced below.

Additional amino acid(s) have been inserted in between the first amino acid downstream of D_2 and the first amino acid upstream of D_3 of said aspartic acid-rich domain.

At col. 5, line 36, please delete "[(DXX(XX)D)]" and substitute "$\underline{(D_1D_2X_1(X_2X_3)X_4D_3)}$" therefore. The paragraph containing this text is reproduced below.

It has been proposed that there are five conserved regions in the amino acid sequence of a prenyl diphosphate synthase (one subunit in the case of a heterodimer) [A. Chem et al., Protein Science Vol. 3, pp. 600-607, 1994]. It is also known that of the five conserved regions, there is an aspartic acid-rich domain conserved sequence I $D_1D_2X_1(X_2X_3)X_4D_3$ (wherein X denotes any amino acid, and the two X's in the parentheses may not be present) in region II. Although there is also an

aspartic acid-rich domain indicated as "DDXXD" in region V, the aspartic acid-rich domain used to specify the modified region of the amino acid sequence of the present invention is the one present in region II, and this domain is termed as the aspartic acid-rich domain I as compared to the aspartic acid-rich domain II present in region V.

At col. 6, line 6, please delete "[(DDXX(XX)D)]" and substitute "$\underline{(D_1D_2X_1(X_2X_3)X_4D_3)}$" therefore. At col. 6, line 8, please delete "[at the position in the N-terminal direction from D of the C-terminal of said aspartic acid-rich domain]" and substitute "<u>one amino acid position downstream of D2</u>" therefor. The paragraph containing this text is reproduced below.

In accordance with the present invention, in the amino acid sequence of a prenyl diphosphate synthase, at least one amino acid residue selected from (a) the amino acid residues in between the amino acid residue located at the fifth position in the N-terminal direction from D of the N-terminal and the amino acid residue located at the first position in the N-terminal direction from D of said N-terminal of the aspartic acid-rich domain $D_1D_2X_1(X_2X_3)X_4D_3$ (wherein X sequence denotes any amino acid, and the two X's in the parentheses may not be present) present in region II, and (b) the amino acid residue located one amino acid position downstream of D2 has been substituted by another amino acid, and/or

At col. 6, line 16, lease delete "[amino acid residues located at the first position in the N-terminal direction from D of the C-terminal and D of said C-terminal]" and substitute "<u>first amino acid downstream of D_2 and the first amino acid upstream of D_3</u> " therefor. The paragraph containing this text is reproduced below.

An additional amino acid(s) have been inserted in between the first amino acid downstream of D_2 and the first amino acid upstream of D_3 of said aspartic acid-rich domain.

At col. 7, line 67, please delete "[biding]" and substitute "<u>binding</u>" therefor. The paragraph containing this text is reproduced below.

It is known that the distance between the sequence of the ribosome binding site (GGAGG and similar sequences thereof) and the initiation codon ATG is important as the sequence regulating the ability of synthesizing protein from mRNA. It is also well known that a terminator (for example, a vector containing rrn PT_1 T_2 is commercially available from Pharmacia) that directs transcription termination at the 3'-end affects the efficiency of protein synthesis by a recombinant.

At col. 9, line 14, please delete "[a]" and substitute "an" therefor. At col. 9, line 16, please delete "[prrenyl]" and substitute "prenyl" therefor. The paragraph containing this text is reproduced below.

By using the method of producing the mutant prenyl diphosphate synthase obtained by the present invention, the mutant prenyl diphosphate synthase derived from an archaea may be created that is more stable and thus easier to handle and that produces prenyl diphosphate. Furthermore, there is also expected a creation of the farnesyl diphosphate-producing mutant prenyl diphosphate synthase that has the property of the prenyl diphosphate synthase prior to mutation (for example, salt stability or stability in a wide range of pH) added thereto.

At col. 10, line 4, please delete "[Geranylaeranyl]" and substitute "Geranylgeranyl" therefor. This text is reproduced below.

Construction of a Plasmid Containing the Gene for Geranylgeranyl Diphosphate Synthase

At col. 10, line 57, please delete "[TATT-31]" and substitute "TATT-3' " therefor. The paragraph containing this text is reproduced below.

Introduction of the mutation (F77Y, T78S, V80I, I84L, 84PS85) was effected using two nucleotides. First, mutation was introduced as mentioned in Example 3 using the oligonucleotide

5'-GTTCTTCATACTTATTCGCTTATTCATGATAG TATT-3' (SEQ ID No: 7) and a transformant was prepared in accordance with Example 4, and furthermore mutation

was introduced into the plasmid thus obtained using the oligonucleotide

5'-ATTCATGATGATCTTCCATCGATGGATCAAGAT-3'

(SEQ ID No: 8).

At col. 11, line 24, please delete "[H2O]" and substitute "$\underline{H_2O}$ " therefor. The paragraph containing this text is reproduced below.

H_2O 5 µl

At col. 11, line 56, please delete "[H2O]" and substitute "$\underline{H_2O}$ " therefor. The paragraph containing this text is reproduced below.

H_2O make to a final volume of 10 µl

At col. 12, line 35, please delete "[ATATCATG-31]" and substitute "ATATCATG-3' " therefor. The paragraph containing this text is reproduced.

F77Y, T78F, H81L: 5'-TATTTCCTTGTGCTTGATG

ATATCATG-3' (SEQ ID No: 11)